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Response of Some Nucleotides *in Vivo* to Gamma Radiation on *Euglena* Cells in Two Different Growth Phases

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A mode of radiation effect was compared by means of the column chromatographic investigation of intracellular nucleotides between logarithmic phase cells and stationary phase ones. Radiation dosage used was 1×10^5 R gamma rays of Co-60. The nucleosides were more easily broken by the irradiation than the nucleotides. The nucleotides increased in quantity after the irradiation in the case of monophosphate, but decreased in quantity in di- and tri-phosphates. This tendency was observed markedly in stationary phase cells in comparison with logarithmic phase cells.

INTRODUCTION

The degree of radiation damage on RNA level of *Euglena* cells varies with the difference of growth phase of the cells.[1] That is, the RNA of stationary phase cells is easily broken down by gamma irradiation in comparison with that of logarithmic phase cells. This difference of radiation effect may be due to the change of molecular structure of RNA or the change of intracellular conditions. Judging from the limited data available in our laboratory, the radiation effect of RNA *in vivo* seems to involve more interesting problem than that of RNA *in vitro*.[2,3] In other words, the change of intracellular conditions in cell growth still seems to play more important role as a cause of difference of the irradiation damage mentioned above.

Observations on the radiation damage of RNA under some various physiological conditions were relatively scarce by the present time. In order to approach this problem, many data have to be accumulated. The present paper deals with the radiation effect of nucleotide level on two different growth phase cells.

MATERIAL AND METHODS

Material and Irradiation

The material used in the present investigation (Experiments I and II) was *Euglena gracilis strain Z*, kindly provided from Dr. Y. Tsubo of Kobe University. The culture flask containing 1500 ml of fresh nutrient solution[4] was used individually. Approximately 5×10^6 cells were transplanted into the fresh nutrient solution and cultured as 28°C in days of different incubation periods. The cul-

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tures of 2 to 6 flasks were used for one experiment. The material cells were gathered centrifugally from the cultures incubated for 4, 10 and 20 days for Experiment I and for 6 and 12 days for Experiment II, respectively. The cells incubated for 6 days were named as logarithmic phase cells and the cells incubated for 12 days stationary phase cells.[1] The cells used per one experiment was approximately 2 to 3×10^8 cells.

The irradiation of the cells was carried out with the Co-60 gamma ray irradiation facility of the Institute for Chemical Research of Kyoto University. The radiation dose used was 1×10^5 R. The dose rate of the facility used 1.16×10^5 R/hr.

Perchloric Acid Extraction and Column Chromatography

Twenty ml of cool 10% perchloric acid solution was added to approximately 2.4×10^8 cells washed thoroughly with distilled water and was preserved at 2°C for one hour. Then the perchloric acid extract separated centrifugally was neutralized with potassium hydroxide solution. This neutralized solution was used as the sample for ion exchange chromatography.

For chromatography, Dowex 1-X8, chloride form (200-400 mesh) was converted into formate form by the procedure of Hurlbert *et al.*[5] The nucleotides were eluted by a modified formic acid and ammonium formate elution procedure of

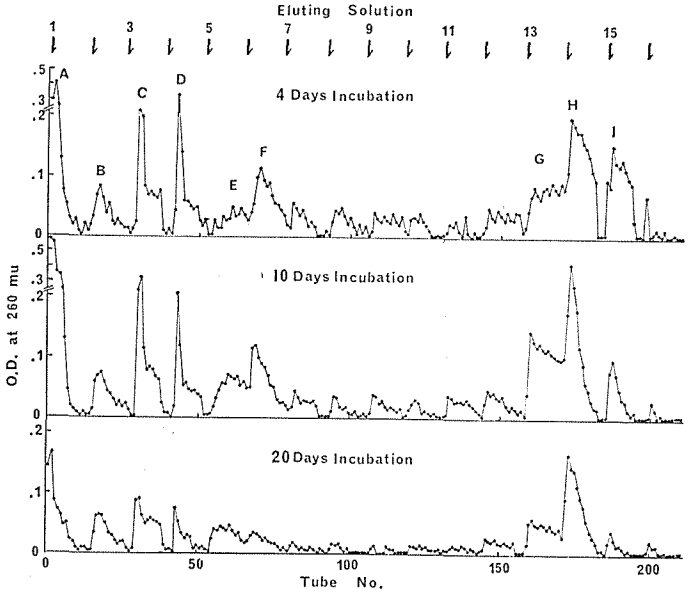

Fig. 1. Chromatographic patterns of nucleotides of non-irradiated cells in three different growth phases.

Eluting solution: (1) distilled water, (2) 0.1 M ammonium formate (Af), (3) 0.2 N formic acid (F), (4) 1.0 N F, (5) 1.5 N F, (6) 2.0 N F, (7) 2.5 N F, (8) 3.0 N F, (9) 3.5 N F, (10) 4.0 N F, (11) 4 N F+0.05 M Af, (12) 4 N F+0.1 M Af, (13) 4 N F+0.2 M Af, (14) 4 N F+0.3 M Af, (15) 4 N F+0.4 N F+0.4 M Af, (16) 4 N F+0.6 M Af.

Cell number: approximately 1.8×10^8 cells in each chromatography.

Cohn and Volkin.[6] All the other procedures for the present chromatography were almost same as those reported previously.[2]

RESULTS

I. Change of Nucleotide Level During Cell Growth

The top of Fig. 1 is a chromatographic pattern of mononucleotides of the early logarithmic phase cells incubated for 4 days. The middle one is that of the late logarithmic phase cells incubated for 10 days. The bottom is of the late stationary phase cells incubated for 20 days.

In each of these chromatographic figures, 9 main fractions obtained were designated by A, B, C····· according to their eluting sequence. The representatives among nucleosides or nucleotides contained in each fraction were listed

Table 1. Nucleotide Composition in the Fractions Separated by Dowex-1 Formate Column Chromatography.

Fraction	A	B	C	D	E	F	G	H	I
Eluting solution	1	2	3	4	5	6	13	14	15
Nucleoside	Gyt*	Gua	CMP	AMP	GMP	UMP	GDP	ATP	GTP
Nucleotide	Uri	Ino							

Cyt; Cytidine, Uri; Uridine, Gua; Guanosine, Ino; Inosine.

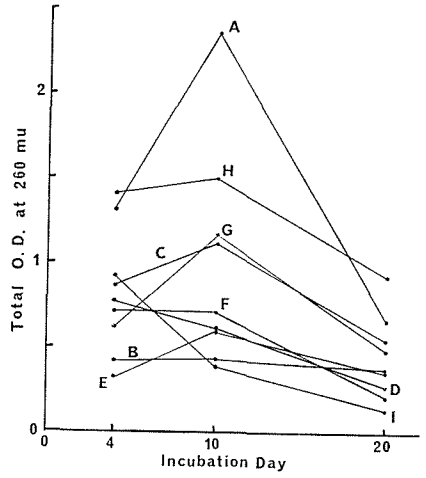

Fig. 2. Relative change of nucleotide composition during the growth of non-irradiated cells.
A to I in this figure are the same as designated in Fig. 1.

in Table 1. The relative change of nucleotide amount of each fraction was shown in Fig. 2. The nucleotide amount of a fraction was calculated with total optical density at 260 mμ of all the effluents of the fraction.

As is shown in Fig. 2, 6 fractions of A, C, E, F, G and H were especially characteristic in size or in change of nucleotide amount, having a peak in the process of the change of their nucleotide amount during cell growth. That is,

the peak was observed in the 10 day incubated cells in every cases. But the nucleotide amount of fractions D and I was the highest in the 4 day incubated cells and then it decreased according to the progress of cell growth. It was noted that the nucleoside fraction B hardly changed in quantity through the whole course of cell growth. The tendency of quantitative change of the 6 fractions mentioned above almost agreed with that of RNA amount during cell growth.[1] Two fractions of D and I reached the peak of nucleotide amount slightly earlier than the case of RNA.

II. Radiation Effect on Intracellular Nucleotides

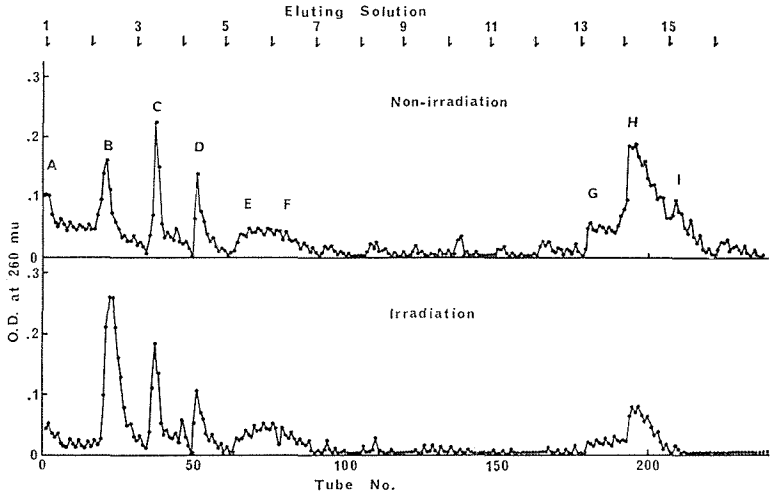

Fig. 3. Chromatographic patterns of nucleotides of non-irradiated and gamma irradiated logarithmic phase cells.
Cell number: approximately 2.4×10^8 cells in each chromatography.
Eluting solution: the same as those shown in Fig. 1.

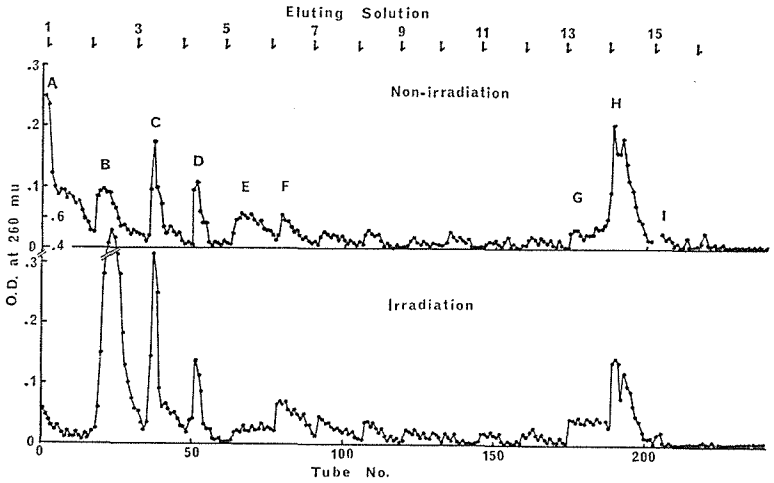

Fig. 4. Chromatographic patterns of nucleotides of non-irradiated and gamma irradiated stationary phase cells.
Cell number and eluting solution were the same as those shown in Fig. 3.

The 1×10^5 R gamma irradiation was carried out on two different growth phase cells, logarithmic and stationary. The results of nucleotide analysis of the irradiated and non-irradiated cells were shown in Figs. 3 and 4, respectively. The fractions were designated also by A, B, C……, corresponding to each each one in a similar way to Fig. 1. From the results of Figs. 3 and 4, the relative quantity of nucleotides in a given fraction was calculated from total optical density of the ultraviolet light absorbing substances in the fraction. Then the irradiation effect on nucleotide level in the present experiment was estimated with the rate (%) of the total optical density mentioned above of the gamma irradiated cells to that of the non-irradiated cells. The results were shown in Table 2.

Table 2. Relative Quantity of Nucleotides of Gamma Irradiated Cells to those of Non-irradiated Ones.

Fraction	A	B	C	D	E	F	G	H	I	Average of A to I	Grand Total
Log cell* (%)	49	174	98	88	103	117	46	33	10	81	66
St cell* (%)	25	411	192	132	53	188	139	70	53	143	124

Log cell: logarithmic phase cells, St cell: stationary phase cells.
Grand total: grand total of optical density at 260 mμ of all the effluents of 230 test tubes.

In the irradiation of the logarithmic phase cells, fraction A decreased in quantity approximately by 50 %, fraction B increased in quantity by 70 %, fractions C, D and E hardly changed in quantity, fraction F increased by 20 %, fractions G, H and I decreased by 50 to 90 %, respectively. These irradiation damages resulted in approximately 20 % decrease in average of all the fractions of A to I. But this value became also to 40 % in average of all the effluents of total 230 test tubes.

From the results mentioned above, it may be suggested that (1) the pyrimidine nucleosides are more easily broken out by the irradiation than the purine nucleosides, (2) the nucleoside than the nucleotides, and (3) the poly-phospho-nucleotides than the mono-phospho-nucleotides, respectively.

In the irradiation of the stationary phase cells, only two fractions of A and E were decreased by approximately 50 % of the value of the irradiated logarithmic phase cells. All the rest fractions were increased by about 2 to 3 times of each value of the irradiated logarithmic phase cells. The increasing ratios of each fraction of the irradiated stationary phase cells to those of the irradiated logarithmic phase cells were 2.4 times in fraction B, 2 times in fraction C, 1.5 times in fraction D, 1.6 times in fraction F, 3 times in fraction G, 2 times in fraction H and 5 times in fraction I, respectively.

From these results stated above, it was assumed that the free nucleosides and nucleotides, resulted from the irradiation damage of intracellular high molecular substances, were increased in quantity in the irradiated cells. Moreover, from the fact that this nucleotide increase was markedly observed in the stationary phase cells, it seems reasonable that the intracellular high molecular substances,

e. g. polynucleotide or RNA, of the stationary phase cells are more sensitively broken out by gamma irradiation than those of the logarithmic phase cells.

DISCUSSION AND CONCLUSION

It is well known that the intracellular component varies with cell growth. The change of nucleotide composition of *Euglena* cells was also observed during the growth of culture. That is, the almost nucleotides *in vivo* increased in quantity from lag phase to logarithmic phase and then decreased in quantity along to the progress of stationary phase. But it seems to be possible that each nucleotide takes its own degree of quantitative change and its own time to reach the peak. Especially two fractions of D containing AMP and I containing GTP reached the peak % at the end of lag phase or at least in the early stage of logarithmic phase. This stage is slightly earlier than the peak of RNA synthesis. Therefore, it may be suggested that the substances containing AMP, GTP *etc.* in fractions D and I must be much accumulated before RNA synthesis in the cells. Of course, the absolute quantities of pyrimidine nucleosides of fraction A. ATP of fraction H or CMP of fraction C were by far more than those of AMP of fraction D or GTP of fraction I. In addition to this, these most nucleotides were increased in quantity corresponding to the period of RNA synthesis. Therefore, in spite of relatively small quantity, it appears that these two fractions of D and I may become a sensitive indicator of the increasing activity for cell growth.

So far as the present data of 260 mμ ultraviolet light absorbing substances, nucleosides and nucleotides extracted with 10 % perchloric acid and separated by the Dowex-1 column chromatography are concerned, the ultraviolet light absorbing substances in the irradiated logarithmic phase cells was decreased in quantity by approximately 20 to 40 % in comparison with that of the non-irradiated cells. Contrary to this, the substances of the irradiated stationary phase cells were increased in quantity by almost similar percentage to that mentioned above. The present result suggests that there is a remarkable difference in a mode of response to gamma radiation between the logarithmic phase cells and the stationary phase ones.

In the case of the logarithmic phase cells, fraction A containing pyrimidine nucleotide and fractions G, H and I containing di- or tri-phospho-nucleotides, respectively, were decreased in quantity over 50 % after the irradiation, but no marked change was recognized in fractions containing mono-phospho-nucleotides. This result agrees with report that the more the number of phosphorus residue in a nucleotide is, the more easily the nucleotide breaks.[7] But the monophospho-nucleotides themselves are not necessarily intact by gamma irradiation. Little change of the fractions containing monophospho-nucleotides, at least in the present investigation may suggest that a decrement portion of the monophospho-nucleotides might be produced by the irradiation was virtually offset with an increment of the monophospho-nucleotides released by the partial degradation of intracellular high molecular substances.

Fraction B containing purine nucleosides of the logarithmic phase cells were increased in quantity over 70 % after irradiation. Purine base may hardly be vulnerable to radiation in comparison with pyrimidine base.

On the other hand, the radiation effect on each fraction separated from the stationary phase cells might be exaggerated in the logarithmic phase cells: the former about 2 times of the latter with respect to the quantitative change of ultraviolet light absorbing substances in the present experiment. In the case of the stationary cells the rest fractions except fraction A were increased in quantity after the irradiation: 4 times in fraction B, 2 times in fractions C and F, 30 % increment in fractions D and G, respectively. This quantitative increase perhaps depends on the radiation damage of the intracellular high molecular substances just same as the case of the logarithmic phase cells. Again, it will be assumed that the intracellular high molecular substances, such as RNA or polynucleotides, of the stationary phase cells are in general more easily broken out by ionizing radiation than those of the logarithmic phase cells. Some similar results have been investigated using *Euglena* cells.[3] But it still remains unexplained why difference in radiation effect developes between logarithmic phase cells and stationary phase ones, in spite of the same molecular structure *in vivo*.

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